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A STUDY OF THE VALUE OF THE LIVING VACCINE IN THE CONTROL OF BOVINE INFECTIOUS ABORTION *

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Since the discovery by Bang of *Bacterium abortus* as the definite cause of bovine infectious abortion this organism and the disease it produces has commanded the attention of investigators and others interested in the cattle industry. Early recognition of the serious economic losses incurred by this disease has led to extensive studies directed toward effective control measures. The previous success of vaccine therapy in the control of certain other infectious diseases stimulated inquiry relative to the control of infectious abortion by the use of biological agents. Although the reported studies represent extensive work by prominent investigators the results are so variable as to leave much doubt as to the relative merits of any particular agent beyond the general statement that no reliable prophylactic or therapeutic agent has yet been found. Brief reference to certain reported investigations carried out on the same general plan as the work here reported is made for purposes of direct comparison.

Hart and Traum[1] in their most recent report on the relation of the subcutaneous administration of the living *Bacterium abortus* vaccine to the immunity and carrier problem of bovine infectious abortion, give the results of their observation on 56 females through two pregnancies. In group No. I, consisting of 20 animals which were vaccinated and later artificially infected, 17 animals became pregnant during both years with no losses due to abortion. In control group No. II (a), comprising 10 animals which were not vaccinated but artificially infected, six of 10 pregnancies terminated in abortions the first year. During the second year, 9 pregnancies resulted in as many normal calves. Control group (b), consisting of 5 non-vaccinated and non-infected females, 10 pregnancies over a two-year period terminated in normal calvings. In group No. III, comprising 10 vaccinated females not arti-

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ficially infected, 4 pregnancies the first year and 8 pregnancies the second year resulted in as many normal calvings. As a result of these observations the authors conclude in part: "The value of the living cultures of *Bacterium abortus* in preventing abortion in vaccinated animals when subjected to identical infection that produced abortion in the control animals was demonstrated."

Buck and Creech[2] report one abortion to 22 pregnancies of 11 animals which had received a subcutaneous injection of 20 cc. of living *Bact. abortus* Bang, and artificial infection after becoming pregnant. In the control group, consisting of 8 non-vaccinated and artificially infected animals, 8 pregnancies ended in 7 abortions. The authors further report the results obtained by the use of the living vaccine in a large dairy herd of 1141 animals of which 772 were vaccinated while the remainder were left as controls. Six hundred and seventeen pregnancies in the vaccinated group terminated in 81 (13.1 per cent) abortions, while 294 pregnancies in the control group terminated in 52 (17.7 per cent) abortions. The second gestation period with a lesser number of animals terminated in 10.3 per cent abortions for the vaccinated and 14.1 per cent abortions for the control group. These figures are only of relative value in that they were based largely upon clinical observations and not carefully controlled bacteriological examinations. In discussing the results obtained under experimental conditions, the authors state, "Considerable encouragement was derived from the results obtained with living abortion organisms."

Smith and Little[3] after extensive studies on vaccinal immunity toward infectious abortion summarize and conclude in part:

"1. In one experiment comprising 134 controls and 53 vaccinated heifers, the abortion rate following vaccination with living cultures was 16.7 and 11 per cent respectively for the first and second pregnancies as compared with 25.1 and 19.2 per cent for the control groups.

"In a second experiment comprising 35 heifers treated with heated cultures and 10 with living cultures, the combined abortion rate for the first pregnancy was 14.7 per cent as compared with 41.6 per cent for 38 controls. The rate for the 10 treated with living cultures was zero. The above

estimates do not include those cases in which full term pregnancies were associated with infected or diseased placentas.

"2. Vaccination with living cultures should be applied only in herds in which abortion in the first pregnancy is frequent and in which cows freshly introduced abort the first or second calf."

"3. Vaccination with small doses of living bacilli (one agar slant or less) practiced two or three months before conception is not a dangerous process."

The experimental work recorded in this paper was projected during the summer of 1922. At that time the results obtained in our study of the immunity induced by the living vaccine under experimental conditions, and artificial infection[4] suggested a detailed observation on the efficiency of this agent under farm conditions and natural infection. The results obtained in both experiments and the conclusions drawn therefrom while not identical are very similar. These results indicate that the exposure to infection under natural conditions was as rigorous as that induced artificially in the experimental herd.

The principal object in undertaking this work was to study the effectiveness of the living vaccine in producing an immunity toward *Bact. abortus* Bang infection. Certain other problems closely associated and of such vast importance as to influence the efficacy and desirability of its use have also been studied. We refer especially to the carrier problem of those animals which are vaccinated; the length of time they harbor infection and the danger of their acting as disseminators of the disease to non-infected animals. Aside from the immunizing value of the living vaccine and the danger of the carrier problem arising from its use, one must also consider its effect upon the reproductive organs and subsequent breeding efficiency if we are to judge of its practical application and merits.

EXPERIMENTAL PROCEDURE

The Herd.—The data here presented are our observations on a total of 60 animals comprising members of a pure bred dairy herd which had suffered losses from abortions for a number of years. Such clinical abortions according to available herd records had ranged from 11.3 to 21 per cent during

the years of 1917 to 1921 inclusive. Many of these abortions were due to *Bact. abortus* Bang infection. This is true because in 1918 when the first sera tests of the herd were made, 72 per cent of the animals gave suspicious or positive reactions and 27.7 per cent gave negative evidence of *Bact. abortus* Bang infection. In 1919, 58.7 per cent of the herd gave a positive reaction while 40.2 per cent were negative. This increase of negative animals over the previous year is explained by the presence of a larger number of heifer calves. The percentage of infection remained approximately the same during these years and the herd may be classified as badly infected, and one in which the effectiveness of an immunizing agent might be judged.

Methods.—At the beginning of the experiment the herd was divided into two groups, equal as to number and per cent showing positive serologic evidence of infection. Due to our endeavor to conduct this work on a practical basis we did not interfere with the normal movement of animals into and out of the herd. This naturally led to the addition of animals to either vaccinated or control groups as occasion required in order that the equality in number be maintained. This addition to the groups also applied to those heifers raised in the herd upon attaining breeding age. All animals were maintained under the same conditions with no attempt at isolation except shortly before and following parturition, a practice which is normally followed in any well managed herd. All of the experimental animals received the same care at parturition and every effort was made to correct the pathologic processes incident thereto by frequent examinations and treatments. Since the herd comprised four of the dairy breeds, a like number of bulls were used throughout the experiment. Frequent serologic tests were run on these animals but always with negative results. Both reacting and non-reacting females were served by the same sire. No special precautionary measures such as douching of the sheath or disinfectant treatment of the buttocks of the female, were practiced. No female was released from quarantine or bred until the genitalia had returned to normal and discharges ceased. All sires used throughout the experiment were potent, with difficult conception and sterility attributable to pathologic changes in the female reproductive organs. In no instance was any animal subjected to artificial infection, beyond that induced by the

use of the living vaccine. The efficiency of the live organisms in producing an immunity toward abortus infection in this report must therefore be judged on the basis of natural herd infection and the extent of this disease in the control group.

Vaccination.—In the preparation of the vaccine considerable care was exercised to select strains of known pathogenicity freshly isolated from a variety of sources. As a rule, four strains isolated from fetal stomach contents, placental material, milk and colostrum were used. In several instances a strain isolated from a testicular infection were also included. The cultures were grown on serum agar media for a period of five days under 10 per cent CO_2 tension. After examining all transfers for purity, the growth was washed with normal saline solution and diluted to a density of tube No. 2 of the MacFarland Nephelometer. To insure the use of viable organisms, the vaccine was always injected within three hours following its preparation. This three-hour period was purely arbitrary and was simply followed in order that the method used and loss in viability may be comparable throughout the experiment. In order to check the possible importance of the quantity of vaccine used, the dosage varied in the amounts of 60, 45, and 20 cc. per individual. All injections were made subcutaneously in the prescapular region. In those individuals comprising the initial group where 60 cc. of the vaccine was injected at a single point, considerable trouble was experienced with swelling and abscess formation. This difficulty was subsequently very largely overcome by multiple injections over a larger area. Beyond a temporary rise in temperature, and in a few animals a short period of malaise, no general disturbance was evident after vaccination. All heifers were vaccinated sixty days prior to being bred. In those instances where cows were added to the vaccinated group, or where an animal was revaccinated, such individuals were vaccinated just as soon as involution had taken place. In no instance was an animal revaccinated unless the serologic tests indicated a decrease in the agglutinins or complement-fixing antibodies.

METHODS OF STUDY

It will be found that the recorded data represents observations resulting from clinical, serological and bacteriological

study of individuals belonging to the vaccinated and control group. The clinical observations comprise such points as: (a), Number of services per pregnancy; (b), Duration of Pregnancy; (c), Number of healthy calves; (d), Number of abortions; (e), Pathologic and physiologic changes at the time of parturition; (f), The breeding efficiency.

The serological observations included the sera tests of all animals in the herd, including heifers of 6 months and bulls used in service. The agglutination and complement-fixation tests were used at least once every 6 months and sometimes with greater frequency as our observations in individual cases seemed to demand. The serum dilutions for the agglutination tests were 1 : 25, 1 : 50, 1 : 100, 1 : 200, 1 : 500, 1 : 1000. During the forepart of the experiment the serum dilutions employed in the complement-fixation test were 0.1, 0.5, and 0.025 cc., which were later (May, 1925) changed to 0.1, 0.04, 0.02, and 0.005.

Bacteriologic Examinations

In view of the fact that abortions may be the result of other causes than infection with *Bact. abortus* Bang it is a matter of utmost importance that a thorough bacteriologic study be made of all fetal material if we are to form an accurate opinion of the efficiency of an immunizing agent. The possible dangers resulting from the use of the living organism as an immunizing agent, in creating carriers of the disease and the presence of the organism in the animal body can only be determined by a bacteriological examination of those excretions and secretions in which *Bact. abortus* Bang is eliminated. The same bacteriologic examinations were made on members of each group regardless of the sereologic evidence of the presence or absence of *Bact. abortus* Bang infection. While a bacteriologic study of the placental membranes, colostrum, and milk of non-reacting individuals may seem superfluous, the results obtained have been such as to contribute valuable data to the carrier problem and control of abortion by the use of the serologic tests.

Bacteriologic examination of fetuses consisted of cultures of the lungs, liver, spleen, kidneys, and contents of the abomasum. In each instance, two guinea pigs were injected with 2.5 cc. of stomach contents obtained from the abomasum.

Where abortions occurred in early pregnancy or where the abortion took place while the animal was at pasture and the fetus was not recovered, the vaginal discharge was examined by culture and guinea pig inoculation. Immediately upon removal or expulsion of the placental tissues the chorion was examined and numerous tufts, especially those showing gross evidence of pathological changes were removed. The material thus obtained was cultured, smear preparations were stained for microscopic examination, while other portions were placed in a sterile mortar and thoroughly triturated with the addition of physiologic saline. Approximately 0.5 to 1.5 cc. of this saline suspension was injected intraperitoneally into each of two guinea pigs.

In order to study the presence of *Bact. abortus* Bang infection in the udder and its elimination therefrom in the vaccinated and control groups, milk samples were obtained from each individual about three times per lactation period. Occasional drying off, sale of the animal, mastitis, and official test work interfered with rigid carrying out of this program. The technique employed in the milk examinations was that described by Fitch and Lubbehusen.[5]

All guinea pigs used for inoculation purposes throughout this experiment were those whose sera had given negative serologic evidence of infection with *Bact. abortus* Bang. The practice is a simple one and eliminates erroneous conclusions due to natural infection.

PRESENTATION OF DATA

A concise and easily understandable presentation of the data at hand is somewhat difficult due to the detailed work involved in the study of the problem and the plan of the experiment which did not interfere with the normal movement of animals into and out of the herd. It will be noted (Charts I and II) that the number of pregnancies per individual vary from one to four, due to the addition of animals to both groups at intervals when replacements were required. The procedure likewise necessitated the vaccination of individuals at irregular intervals as they became available for use. To assist in presenting the collected data in a form which will aid in a ready interpretation of the results, and claim the interest of the clinician as well as the bacteriologist and immunologist

we have abandoned an extended protocol treatment, and have resorted entirely to the use of charts. We have further endeavored to group the data into three divisions, namely: (a) Clinical observations; (b) Bacteriologic findings; (c) Serologic reactions. Each embraces a field of interest and it is only by correlating the data thus obtained that final conclusions can be drawn.

The clinical observations (Charts I and II) present: (a), the quantity of vaccine used and dates of vaccination; (b), Number of pregnancies; (c), Number of services per pregnancy; (d), Dates of normal calvings; (e), Dates of abortion; (f), Length of gestation period; (g), Retention or expulsion of fetal membranes; (h), Present condition, with general remarks.

The Data included in the bacteriologic findings (Charts III and IV) comprise: (a), Dates of normal calvings; (b), Dates of abortions; (c), Results of the bacteriologic examination of fetuses, placenta, and milk. In the latter is included the number and dates of examination.

The results of the serologic tests (agglutination and complement-fixation) on the vaccinated and control groups, together with dates and reaction of the dam, where possible, are all included on Chart V.

SUMMARY OF THE CLINICAL DATA

Vaccinated Group

There are presented in this group a total of 51 pregnancies of which 42 are terminated at the present writing. Of this number 34 cows (or 81 per cent) calved normally, while 8 (or 19 per cent) aborted. With the exception of animals 533 and 534 that aborted on the 112th and 156th day respectively, abortions occurred between the 180th and 244th day of gestation with an average of 198 days. There were 131 services required for 51 pregnancies or an average of 2.55 per pregnancy. The average number of pregnancies for the 27 animals under consideration was 1.88. The fetal membranes were expelled following 29 (or 69.5 per cent) and retained following 13 (or 30.9 per cent) of the pregnancies. Of those animals that calved normally, 17.64 per cent suffered from

retention of the fetal membranes, while 87.5 per cent of those that aborted were affected in like manner.

Control Group

In this group there were a total of 79 pregnancies, 66 of which are terminated at the present time. Of the 66 terminated pregnancies, 47 cows (or 71.2 per cent) calved normally while 19 (or 28.7 per cent) aborted. In two instances, animals Numbers 8 and 138, abortions occurred at approximately two months, after each had been declared pregnant by absence of œstrum and physical examination. The remaining 17 abortions occurred between the 145th and 255th day of gestation with an average of 209 days. Seventy-six pregnancies (3 animals were pregnant when purchased) required a total of 195 services or an average of 2.56 per pregnancy. The average number of pregnancies per animal was 2.39. The fetal membranes were expelled following 44 (or 66.6 per cent) of the pregnancies. Of the animals that calved normally 17 per cent suffered from retention of the fetal membranes while 73.7 per cent of those which aborted were similarly affected. It is of further interest to note that only 8.5 per cent of the animals that calved normally and suffered from retention of the fetal membranes failed to show serologic evidence of *Bact. abortus* Bang infection.

DISCUSSION OF THE CLINICAL DATA

The general use of many immunizing agents has and will largely depend upon clinical manifestations of efficiency, with the result that the proper interpretation of clinical facts gathered in work of this kind is not only necessary, but is many times of far-reaching importance. The degree in which the clinical data and conclusions drawn therefrom can be supported by a bacteriologic study, is variable. It may be said that final conclusions can only be drawn by a correlation of the clinical and bacteriologic findings. Although it was our intention at the onset of the experiment to collect data on an equal number of pregnancies from each group, it will be noted that the pregnancies presented by the vaccinated group are considerably less than those of the controls. It will be noted that this inequality is not due to a lower breeding efficiency

but to the disposal of animals by sale, reaction to the tuberculin test, and death. In order, therefore, that the results obtained in the two groups might be directly comparable, the data must be considered on a percentage basis. The vaccinated group presents 8 or 19 per cent abortions out of 42 terminated pregnancies. Cow 140 is the only animal that aborted twice. The control group represents 66 terminated pregnancies, 19 (or 28.7 per cent) of which resulted in abortions. Four of the animals; namely, 134, 138, 374, and 537, aborted twice. These figures not only show a larger per cent of abortion in the control group but the percentage of animals involved is greater. Of the five animals that aborted twice, 4 (or 80 per cent) belong to the control group.

The retention of the fetal membranes is always to be viewed as a manifestation of placentitis, often of bacterial origin. While a number of bacteria have the power of invading the placental tissues, this condition is so commonly associated with *Bact. abortus* Bang as to be viewed by many as symptomatic of infection with this organism. In the vaccinated group the fetal membranes were retained in 30.9 per cent, and the control group in 33.3 per cent of the cases. The close association of retained placenta and abortion may be gathered from the fact that 87.5 per cent of the vaccinated and 73.7 per cent of the control animals that aborted retained their placentas. This figure would have been still higher were we to eliminate from our calculations those abortions occurring before the third month of gestation. That retention of the fetal membranes following apparently normal calvings is fairly constant may be concluded from the fact that 17.6 per cent of the normal calvings in the vaccinated group and 17 per cent in the control group were so affected. Just what percentage of these retentions are due to infections with *Bact. abortus* Bang will be discussed in connection with elimination of the organism. That retention of the fetal membranes may and does occur without infection with the Bang organism is evident in that 8.5 per cent of the animals that calved normally and retained the fetal membranes failed to show serologic evidence of *Bact. abortus* Bang infection. According to our observations, the assumption that retention of the fetal membranes is indicative of infection with *Bact. abortus* Bang would probably be correct in approximately 90 per cent of the cases.

A fair estimate of the breeding efficiency of the vaccinated and control group may be obtained by comparing the average number of services per pregnancy and average number of pregnancies per animal. Inasmuch as the same proven sires were used upon members of both groups, and all animals received proper treatment as occasion required, failure to conceive was due to temporary or permanent pathologic changes in the female genitalia.

The average number of services per pregnancy in the vaccinated group was 2.55 as compared with 2.56 for the controls. Despite the fact that both groups appeared to conceive with equal readiness, the average number of pregnancies was 1.88 and 2.39 for the vaccinated and control groups respectively. This seeming discrepancy may be explained by the fact that a number of the animals of the vaccinated group were disposed of when "open" following the termination of only one pregnancy.

Since animals in both groups seem to balance in their ability to conceive, and both suffer in approximately the same percentage from temporary pathologic involvement of the genitalia, the efficiency of the living vaccine from a clinical viewpoint must be judged by the decrease in the number of abortions and the probably increase of permanent pathologic changes in the genitalia attributable to its use. A review of the clinical data of the vaccinated group shows that animals 363, 523, and 355 were sold as sterile. After calving twins on August 18, 1925, cow 363 developed metritis, which became chronic. The development of adhesions involving tubes and ovaries together with a number of small abscesses rendered the animal sterile. Cow 355 calved normally on September 6, 1924. The fetal membranes were expelled one hour after calving and involution took place in a normal manner. She was bred on September 29, October 23, and November 12, 1924. A persistent *corpus luteum* was removed from the left ovary on January 12, 1925. She was bred four days later. On March 13, 1925, a *corpus luteum* was removed from the right ovary, Estrum appeared two weeks later, but continued breeding failed to result in conception. After four services she was again examined and found to be affected with salpingitis and cystic changes of the left oviduct and ovary. Removal of the affected ovary and tube was performed. Recovery was uneventful and the cow was again rebred a

number of times without success. She was then sold for slaughter. Cow 523 was a large coarse heifer with masculine tendencies and was temporarily sterile as a heifer. After numerous services she conceived and on January 2, 1923, she calved normally. The fetal membranes were promptly expelled and involution progressed normally. Later, cystic changes occurred in the right ovary. The cysts were ruptured on Jaunary 15th, and again on the 26th of January. An examination of the reproductive organs on February 21, 1923, showed all organs to be apparently normal. This cow was then bred seven times without successful conception. On account of reduced fertility together with the gradual increase in masculine appearance she was sold for slaughter. In the control group, only one animal, 142, was sold as sterile. Sterility in this instance resulted from cystic degeneration of the ovaries. Although this animal was a strong positive reactor, and suffered from abortion on the 220th day of gestation, bacteriological examination failed to show the presence of *Bact. abortus* Bang in either the fetus or placental membranes.

We desire to call attention to animal 101 as being temporarily and possibly permanently sterile, although she is being retained in the herd and is now undergoing treatment with the hope she will again calve successfully. This cow is an aged Jersey which has produced twelve strong and vigorous calves which stamps her as a wonderful breeder. This record is especially significant on account of the fact that she has for a greater portion of her life been continuously exposed to abortion infection. At her last calving date, December 8, 1924, she produced a strong, vigorous calf but suffered partial retention of the fetal membranes. The existing placentitis developed into an extreme metritis involving mainly the left cornu, oviduct, and ovary. Recovery was rather slow and resulted in fairly extensive adhesions involving the left oviduct and ovary. This cow reacted on numerous occasions to the serologic tests, but was never strongly positive and on one occasion gave a negative reaction. The presence of *Bact. abortus* in the excretions or secretions of this cow has never been demonstrated.

In considering the cases of sterility in the vaccinted group, and their probable relationship to the use of the living vaccine, it is of interest to note that the organism was not

recovered in a single instance, either following normal calving or in the discharges incident to a metritis.

SUMMARY OF BACTERIOLOGIC DATA

Vaccinated Group

A study of Charts III and IV shows that the bacteriologic data comprises examinations of fetuses, placentas, and frequently of milk samples for the presence of *Bact, abortus* Bang infection. As has been stated, this study has embraced both the direct culture method and guinea pig inoculation. In order that the data may be condensed and the basis for our interpretation of the bacteriological findings more readily understood the results are presented in the following table:

TABLE I

Summary of the Bacteriologic Data of the Abortions in the Vaccinated Group (Eight Abortions)

Animal	Fetus	Placenta	Discharge	Milk	Reaction	Remarks
364	—	—	..	—	+ + + — — —	Not due to *Bact. abortus* Bang.
140	—	+	..	..	+ + + + + +	Due to *Bact. abortus* Bang.
520	+	+	..	..	+ + + + + +	Due to *Bact. abortus* Bang.
531	+	+	..	+	+ + + + + +	Due to *Bact. abortus* Bang.
534	..	+	..	—	+ + + + + +	Due to *Bact. abortus* Bang.
140	..	+	..	..	+ + + + + +	Due to *Bact. abortus* Bang.
361	..	..	..	+		Questionable. Probabaly due to *Bact. abortus* Bang.
533	..	..	—	..	+ + + + + +	Questionable. Very early abortion.

Of the 4 fetuses examined 2 were positive while 2 were negative for *Bact. abortus* Bang infection.

Of the 6 placentas examined 5 were positive while 1 was negative for *Bact. abortus* infection.

Two of the four animals examined eliminated the organism in the colostrum or during the lactation following abortion.

Referring to the bacteriologic study of the placenta of normal calvings, we find that two of the 27 examined, or 7.4 per cent, showed the presence of *Bact. abortus* Bang. Considering the elimination of the organism from the entire vacci-

nated group, we find that 7 or 26 per cent of the animals eliminated *Bact. abortus* Bang in the milk while in 6 or 22.2 per cent the organism was found in the placenta or uterine discharges, with a total of 30 per cent of the animals acting as spreaders of infection by elimination of the organism from one or both channels.

Control Group

The outline of our bacteriologic findings and interpretation of the results is as follows:

TABLE II

Summary of the Bacteriologic Data of the Abortions in the Control Group (Nineteen Abortions)

Animal	Fetus	Placenta	Discharge	Milk	Reaction	Remarks
134	+	+	..	—	+ + + + + +	Due to *Bact. abortus* Bang.
134	+	..	+	—	+ + + + + +	Due to *Bact. abortus* Bang.
138	+	+	..	+	+ + + + + +	Due to *Bact. abortus* Bang.
153	+	+	..	..	+ + + + + +	Due to *Bact. abortus* Bang.
360	+	+	..	+	+ + + + + +	Due to *Bact. abortus* Bang.
374	+	+	..	+	+ + + + + +	Due to *Bact. abortus* Bang.
375	+	+	..	..	+ + + + + +	Due to *Bact. abortus* Bang.
521	+	+	..	—	+ + + + — —	Due to *Bact. abortus* Bang.
528	+	+	..	+	+ + + + + +	Due to *Bact. abortus* Bang.
537	+	+	..	—	+ + + + + +	Due to *Bact. abortus* Bang.
537	+	+	..	+	+ + + + + +	Due to *Bact. abortus* Bang.
142	—	—	..	+	+ + + + + +	Not due to *Bact. abortus* Bang.
143	—	—	—	..	— — — — — —	Not due to *Bact. abortus* Bang.
152	—	—	..	—	— — — — — —	Not due to *Bact. abortus* Bang.
368	—	—	..	—	— — — — — —	Not due to *Bact. abortus* Bang.
377	—	—	..	—	— — — — — —	Not due to *Bact. abortus* Bang.
8	..	..	..	—	+ + + + + +	Questionable. Very early abortion.
138	..	..	..	+	+ + + + + +	Probably due to *Bact. abortus* Bang.
374	..	—	..	+	+ + + + + +	Probably due to *Bact. abortus* Bang.

Of the 16 fetuses examined 11 were positive and 5 were negative for *Bact. abortus* infection.

Of the 17 placentas examined 11 were positive and 6 were negative for *Bact. abortus* infection.

Eight of the animals eliminated *Bact. abortus* Bang in the colostrum or during the lactation following abortion.

In this group, the bacteriologic examinations of the placentas of normal calvings demonstrated the presence of the organism in 4 or 12.1 per cent of a total of 33 examined. In this group 8, or 24.2 per cent, of the animals eliminated *Bact. abortus* Bang in the milk while the organism was recovered in the placentas of 12, or 36.3 per cent, of the animals. Forty-two and four-tenths per cent of all members of the group eliminated *Bact. abortus* Bang and thus acted as spreaders of infection.

DISCUSSION OF THE BACTERIOLOGIC DATA

The importance of the bacteriologic data in a study of this kind and the influence of such findings upon the final interpretation of the results obtained is of two-fold importance: First, in determining what per cent of abortions are actually due to the Bang organism; and, second, regardless of the serologic reaction of the individual they serve as the only means by which the carrier and spreader of infection may be detected.

It is unfortunate that circumstances prevented the direct cultural examination of four of the eight aborted fetuses of the vaccinated group. However, inasmuch as the act of abortion is induced not by infection of the fetus proper, but by pathologic changes in the placental membranes, the examination of and demonstration of the organism in the latter tissue is of primary importance. As will be noted in the outlines of examinations made we have been governed in our diagnoses by the presence or absence of infection of the placenta with *Bact. abortus* Bang. Of the 8 abortions occurring in the vaccinated group, 5 (or 62.5 per cent) were due to *Bact. abortus* infection; of the remaining 3 (or 37.5 per cent) one, cow 364, showed no evidence of Bang infection. In another, cow 533, the abortion occurred so early in pregnancy that the results obtained were very questionable, while in the third, cow 361, the abortion was viewed as probably resulting from infection with *Bact. abortus* Bang. The reason for this contention is based upon positive evidence of an active focus

udder of infection in the body and the knowledge that this individual had always been a strong positive reactor to the serologic tests.

Sixteen fetuses of the 19 abortions occurring in the control group were examined by direct culture, 2 were lost while another was too badly decomposed for examination. Of the 16 fetuses examined 11 showed positive and 5 negative evidence of infection with the Bang organism. Since the examination of the placenta checked in all instances with the results obtained by fetal culture, it may be stated with safety that 11 abortions were due to *Bact. abortus,* while 5 were not. Our knowledge of the causitive agent of the 3 remaining abortions is indefinite. Two of the abortions occurring in cows 138 and 374 were very probably due to Bang infection. This contention is supported by our knowledge of an active focus (udder) of infection in both animals and that one had aborted and the other later aborted. A survey of the bacterologic data thus far presented serves to emphasis two important and generally recognized points; namely, that all abortions are not due to the Bang organism, and the consequent probability of error in judging the efficiency of an immunizing agent solely on clinical manifestations. While no bacteriological technic, especially in a study of this kind, is infallible, it is the only safe method by which to determine the degrees of immunity produced. The final result, therefore, upon which the degree of immunity may be judged is 5 (or 11.9 per cent) abortions for the vaccinated group as compared to 11 (or 16.6 per cent) for the controls. These percentages do not include those abortions of questionable classification which were 4.7 per cent and 4.5 per cent respectively of the vaccinated and control groups. While the exposure of vaccinated animals to infection may have been open to question, we believe that the abortion rate and spread of infection in the control group, together with our definite knowledge that 36.6 per cent of all animals eliminated the organism, is sufficient evidence to conclude that the decrease in the number of abortions in the vaccinated as compared with the control animals is an index of the immunity induced rather than lack of exposure.

Abortions Due to Other Causes

Six, or 22.2 per cent, of a total of 27 abortions failed to show *Bact. abortus* Bang as the etiologic factor by a bacterio-

logic study, and 3, or 11.9 per cent, of the cases failed to show evidence of the Bang infection by serologic reaction. Although the recorded data refers only to results of the bacteriologic examinations for *Bact. abortus,* an extensive study was made especially of those abortions which occurred in animals giving negative serologic evidence of infection. Bacteriologic results, as well as examinations for probably mucor or vibrio involvement failed to reveal the causitive agent. Inasmuch as inquiry into herd management and physical examinations were likewise negative, we must, for the present, classify these abortions as occurring without demonstrable cause.

The Carrier Problem

The use of a viable organism, especially those of proven pathogenecity, as an immunizing agent, has always been viewed with misgiving, due to the ever-present danger of spread of the disease to non-infected individuals. This danger has limited the use of the vaccine to those herds showing a high percentage of natural infection. If the degree of immunity produced by the use of the living vaccine depends upon an ever-present low grade active infection the successfully immunized animal must be regarded as an ever-present source of danger as a spreader of infection. In order to compare this danger of the vaccinated as compared with the naturally infected animal a survey of the isolation of the organism from both groups is of interest. Of the 27 placentas examined following 34 normal calvings in the vaccinated group, 2, or 7.4 per cent, cows 136 and 366, showed the presence of the organism, as compared with 4, or 12.1 per cent, of 33 examinations of normal calvings in the control group. Including the number of isolations of *Bact. abortus* from the placentas of the abortions of each group we find that the organism was recovered in 22.2 per cent in the vaccinated as compared with 36.3 per cent of the control animals. The possibility of infection of the placental tissues with *Bact. abortus* in those cases where calving is apparently normal, even though the percentage may be small, serves to emphasize the need of isolation of all animals at the time of parturition.

While the spread of abortion infection through the uterine discharges may be successfully controlled by proper quarantine measures, the danger of milk-borne infection and its con-

trol is less clearly understood. Many investigators are of the opinion, based on experimental results, that calves fed upon infected milk do not harbor the organism for long periods of time and that permanent infection does not take place until sexual maturity. Whatever the danger of milk-borne infection may be, it is of interest to note that the number of animals eliminating the organism through this channel are not materially increased by the administration of the living vaccine, which shows 26 per cent as compared with 24.2 per cent for the control group animals giving off the organisms in the milk. A summation of the bacteriologic results shows that 30 per cent of the vaccinated and 42.4 per cent of the control animals eliminated *Bact. abortus* Bang by either one or both channels and thus acted as spreaders of the infection.

Before concluding the discussion of the carrier problem, we wish to refer briefly to the isolation of *Bact. abortus* Bang from those animals which fail to give positive evidence of infection to one or both of the serologic tests. While this condition is rarely met with, due possibly to disregard of negative animals, it is of sufficient importance to merit the attention of those who hope to succeed in controlling this disease solely by means of the serologic tests. It should be clearly understood that this data is not presented in any effort to discredit the serologic tests, but merely to call attention to their limitations, for we believe that successful control measures must be based upon a correct interpretation of a number of serologic reactions of each herd individual. Animal 366 was vaccinated July 12, 1923. She conceived at the fourth service and calved normally on January 30, 1925. On October 25, 1923, the agglutinin titre was partial at 1: 200, with complement fixation complete at 0.1, 0.5, and 0.025. On September 9, 1924, the agglutinin titre had dropped to a partial at 1: 25 with complement fixation still complete at 0.1, 0.05, and 0.025. On January 28, 1925, two days before the calving date, the agglutinin titre was negative throughout, while fixation remained positive. At parturition on January 30, 1925, *Bact. abortus* Bang was isolated from the placenta. Cow 143, giving a *partial* agglutinin titre at 1: 25 and 1: 50 on October 28, 1922, and thereafter negative to both tests, was found to be eliminating the organism in her milk on October 3, 1924. *Bact. abortus* was also isolated from her placenta following a normal calving on January 4, 1925. It is of interest to note

that this animal aborted during the following pregnancy but no evidence could be found indicating that the Bang organism was concerned. Animal 21 was found to be eliminating the organism in her milk on January 21, 1925. She was and had always been a negative reactor to both of the sera tests. Similar observations have been made by other workers. Hart and Traum[1] refer to such observations in their conclusions with the statement, "A correlation of the agglutination tests of the animals with the definite periods when *Bacterium abortum* was known to have been eliminated shows that this organism may be discharged from the body without its presence being suspected from the agglutination titre of the blood."

CONCLUSIONS

1. Although some degree of immunity toward *Bact.* abortus Bang is induced by the use of the living vaccine, it does not reduce the abortion rate to a desirable minimum and should never be used in other than badly infected herds.

2. Individual susceptibility to *Bact. abortus* Bang infection is extremely variable.

3. The use of the living vaccine did not increase the number of animals which eliminated *Bact. abortus* Bang.

4. Some animals may eliminate *Bact. abortus* Bang without showing serologic evidence of infection.

5. We have been unable to demonstrate that vaccination retards conception nor that increased sterility is directly associated with the use of the living vaccine.

We desire to have it clearly understood that in conclusions 3 and 5 the comparisons are between vaccinated animals and a group naturally infected with *Bact. abortus* Bang. Our experimental herd free of infection with the Bang organism, shows a much higher breeding efficiency than the vaccinated group. In our judgment the only way with our present knowledge to successfully control the disease resulting from infection with *Bact. abortus* is on the basis of the clean herd as determined by the serum tests.

REFERENCES

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3. Smith, T., and Little, R. B., Studies in Vaccinal Immunity Towards Disease of the Bovine Placenta Due to Bacillus Abortus, *Monograph 19, Rockefeller Inst. for Med. Res.*, 1923.
4. Fitch, C. P., and Boyd, W. L., Studies of the Value of Vaccine and Bacterins in Immunizing Cattle to Bact. Abortus (Bang), *J. Amer. Vet. Med. Assn.*, 1924, lxv, 407.
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Clinical Data on Vaccinated Group

Animal No.	Date of Vaccination	Amount of Vaccine	No. of Pregnancies	No. of Services	Calved Normally	Aborted	Duration of Pregnancy	Fetal Membranes	Present Condition	Remarks
12	10/31/22 1/26/25	20 cc. 45 cc.	3	2 1 2	12/ 4/23 10/26/24 1/ 8/26		280 days 266 days 279 days	Expelled Expelled Expelled	Open	
135	1/15/23	30 cc.	1	1	12/11/22		270 days	Expelled		Sold 10/26/23.
136	1/15/23	60 cc.	2	1 4	1/14/24 1/30/25		279 days 278 days	Expelled Expelled		Sold 11/6/25.
140	3/ 7/23	60 cc.	4	1 1 1 1	1/24/24	12/16/24 8/11/25	278 days 201 days 173 days	Expelled Retained Retained	Pregnant	
144	1/ 8/23	60 cc.	2	1 2	1/16/24 (Calf born dead) 3/31/25		282 days 277 days	Expelled Expelled		Sold 5/21/25.
145	1/ 8/23	60 cc.	3	3 2 1	3/20/24 5/ 4/25		284 days 266 days	Expelled Expelled	Pregnant	
310	10/31/22 10/15/24	20 cc. 45 cc.	2	1 7 7	12/ 4/24 5/ 2/25		283 days 278 days	Expelled Retained	Open	
351	10/31/22	45 cc.	2	1 2	10/13/22 10/17/23 (twins)		285 days 256 days	Expelled		Died 10/17/23 at calving.
352	1/ 7/23 10/15/24	60 cc. 45 cc.	2	3 6 0	5/12/24 10/23/25		274 days 293 days	Expelled Expelled	Open	
361	10/31/22 4/21/25	20 cc. 45 cc.	3	3 2 1	3/13/24	7/ 3/25	282 days 207 days	Expelled Retained	Pregnant	
362	3/ 7/23	60 cc.	1	6	11/ 7/ 24		280 days	Expelled		Sold 2/5/25.
363	3/ 7/23 10/15/24 4/21/25	60 cc. 45 cc. 45 cc.	1	4 5	8/18/24 (twins)		268 days	Retained	Sterile	Sold 10/10/25.
364	3/ 7/23 1/ 6/25	60 cc. 45 cc.	3	1 1 1 0	 10/27/24 12/14/25	1/12/23	181 days 278 days 280 days	Retained Expelled Expelled	Open	
520	10/31/22	20 cc.	3	1 4 2 2	10/22/23 1/16/25	 11/10/25	93 days 285 days 244 days	Expelled Retained Retained	Open	
523	3/ 7/23	60 cc.	1	4 7	1/ 2/23		283 days	Expelled	Sterile	Sold 3/6/24.
527	1/15/23	60 cc.	1	5 0	4/20/24		279 days	Expelled		Open when killed due to fracture 11/20/24.
132	7/30/23	45 cc.	2	3 6	1/16/25		288 days	Expelled		Pregnant when killed, 10/30/25.
355	7/30/23 10/15/24 4/21/25	45 cc. 45 cc. 45 cc.	1	2 10	11/ 6/24		276 days	Expelled	Sterile	Sold, 1/25/26.
366	7/12/23	45 cc.	1	4	1/30/25		281 days	Expelled		Sold, 2/4/25.
375	7/30/23	45 cc.	1	7	8/13/24 (twins)		269 days	Retained		Died 9/22/24. Mastitis-Metritis.
511	10/18/23 1/ 6/25	45 cc. 45 cc.	2	1 1 2	10/28/24 12/ 9/25		287 days 283 days	Expelled Expelled	Open	
373	1/31/24 4/21/25	45 cc. 45 cc.	2	2 2	3/ 3/25		267 days	Retained	Pregnant	
531	1/31/24	45 cc.	2	1 12		10/24/24	183 days	Retained	Pregnant	
378	4/23/24 4/21/25	45 cc. 45 cc.	1	3 0	2/15/26		280 days	Expelled	Open	
379	4/23/24	45 cc.	1	1 1	6/17/25		280 days	Expelled	Open	
533	1/ 6/25	45 cc.	2	1 3		5/25/25	112 days	Expelled	Pregnant	
534	1/ 6/25	45 cc.	2	1 2		8/26/25	156 days	Retained	Pregnant	

CHART NO. II

Clinical Data on Control Group

Animal No.	No. of Pregnancies	Services per Pregnancy	Calved Normally	Aborted	Duration of Pregnancy	Fetal Membranes	Present Condition	General Remarks
8	2	3 2	6/14/23	2/ /24	297 days 2 mo.	Expelled Expelled		Sold on 6/22/24.
101	3	1 1 1 10	1/28/23 1/23/24 12/ 8/24		283 days 274 days 283 days	Expelled Expelled Retained	Open	Suffered from metritis following her last calving.
132	1	1	3/11/23		276 days	Expelled		Transferred to vaccinated group 8/30/23.
134	2	1 2		4/ 1/23 12/ 7/24	167 days 165 days	Retained Retained		Killed 3/6/25. Tumor involvement.
138	5	1 1 1 3 1	1/23/23 7/12/25	 10/12/23 3/ 1/24	278 days 224 days 2½ mo. 282 days	Expelled Retained Expelled Expelled	Pregnant	
141	3	1 1 2 3	6/13/23 7/13/24 6/25/25		297 days 280 days 279 days	Expelled Expelled Expelled	Open	
142	1	1 3		3/29/23	220 days	Retained		Sold as sterile, due to cystic ovaries 9/8/24.
143	3	2 1 3 1	11/ 4/23 1/ 4/25	 12/10/25	281 days 285 days 243 days	Expelled Expelled Expelled	Open	
152	3	? 2 7	12/ 8/23	2/28/25	271 days 244 days	Expelled Retained	Pregnant	Pregnant when purchased on 5/6/23. Suffered from metritis at last calving.
153	2	1 1 0	12/ 9/24	12/31/25	281 days 247 days	Expelled Retained	Open	
154	2	3 7 0	5/15/24 1/12/26		284 days 279 days	Expelled Expelled	Open	
350	3	1 2 1 0	11/12/23 3/10/23 1/ 9/26		280 days 280 days 280 days	Expelled Expelled Expelled	Open	
354	2	1 4	12/ 7/22 6/11/24		283 days 280 days	Expelled Expelled		Reacted to tuberculin test. Slaughtered 5/27/25.
358	3	3 1 1 1	8/ 4/23 10/22/24 9/ 4/25		281 days 278 days 277 days	Expelled Retained Retained	Open	
360	3	3 3 4	1/ 4/24	1/26/25	281 days 198 days	Expelled Retained	Pregnant	
368	2	4 2 2	3/ 8/25	12/ 1/25	281 days 228 days	Expelled Expelled	Open	
374	4	2 2 1 2 1	3/ 8/23 3/20/24	 12/13/24 10/21/25	280 days 279 days 152 days 205 days	Expelled Expelled Retained Retained	Open	Suffered from metritis following each abortion.
375	1	?		1/28/23	145 days	Retained		Transferred to vaccinated group 7/30/23.
376	3	1 3 2	2/16/24 5/20/25		276 days 283 days	Expelled Expelled	Pregnant	
377	3	1 3 1	 2/15/25	1/30/24	201 days 279 days	Retained Expelled	Pregnant	
521	2	10 3 0	 2/ 7/26	11/ 5/24	227 days 289 days	Retained Expelled	Open	
524	2	8 1	12/13/24 11/ 3/25		291 days 285 days	Expelled Expelled		Suffered from actinomycosis. Slaughtered 11/16/25.
526	3	1 1 5	3/ 7/24 4/15/25		283 days 284 days	Expelled Expelled	Pregnant	
528	3	? 2 2 2	 2/10/24 11/14/25	2/ 8/23	6½ mo. 281 days 287 days	Retained Retained Retained	Open	
529	2	7 5 1	9/17/24 10/26/25		273 days 283 days	Retained Expelled	Open	
535	2	3 3	1/ 3/25		275 days	Expelled	Pregnant	
536	2	2 3	6/ 2/25		283 days	Expelled	Pregnant	
537	3	2 5 5		11/11/24 8/ 5/25	260 days 236 days	Expelled Retained	Pregnant	
19	2	2 3 0	8/26/24 12/ 5/25		279 days 277 days	Expelled Expelled	Open	
21	2	2 4	12/22/24		294 days	Retained	Pregnant	
369	2	3 6	11/30/24		286 days	Expelled	Pregnant	
370	2	4 1	3/16/25		285 days	Expelled	Pregnant	
372	2	3 2	3/ 1/25		283 days	Retained	Pregnant	

CHART NO. III

Bacteriologic Data on Vaccinated Group

Animal No.	Calved Normally	Aborted	Examination for the presence of Bact. Abortus Bang in:						General Remarks
			Fetus		Placenta		Milk		
			Result	Remarks	Result	Remarks	Result	Date	
12	12/ 4/23				Negative		Negative	12/28/23	
							Negative	4/16/24	
	10/26/24				Negative		Negative	1/21/25	
							Negative	7/29/25	
	1/ 8/26				Negative				
135	12/11/22					Not Obtained	Negative	1/16/23	Vaccinated after the calving date but before milk samples were obtained.
							Negative	7/10/23	
136	1/14/24				Positive		Negative	2/ 5/24	
							Negative	6/12/24	
							Negative	10/ 3/24	
	1/30/25				Negative		Negative	3/28/25	
							Positive	7/25/25	
140	1/21/24				Negative		Negative	1/ 9/24	
							Negative	6/20/24	
							Positive	10/ 8/24	
		12/16/24	Sterile		Positive		Dried		
		8/11/25		Decomposed	Positive		Pigs died	12/15/25	
144	1/16/24				Negative		Negative	2/ 9/24	
							Negative	6/20/24	
							Positive	10/ 8/24	
	3/31/25				Negative				Slaughtered 5/21/25
145	3/20/24				Negative		Negative	5/21/24	
							Negative	10/ 8/24	
							Negative	1/28/25	
	5/ 4/25					Consumed	Negative	6/15/25	
							Negative	9/ 5/25	
310	11/ 4/23				Negative		Negative	12/19/23	
							Negative	4/10/24	
							Negative	8/ 1/24	
	5/ 2/25					Retained	Negative	7/22/25	
351	10–13–22					Consumed	Negative	11/ 1/22	
							Negative	3/28/23	
							Negative	8/29/23	Died as the result of traumatic pericarditis.
	11/17/23					Retained			
352	5/12/24				Negative	Pigs died	Negative	8/ 1/24	
							Negative	2/18/25	
	10/23/25				Negative		Negative	11/10/25	
361	3/13/24				Negative		Negative	4/30/24	
							Negative	8/27/24	Guinea Pigs inoculated with fetal stomach contents died 18 hours afterward.
		8/ 7/25		Decomposed		Retained	Positive	3/28/25	
							Negative	7/22/25	
362	11/ 7/24				Negative				Sold 2/5/25.
363	8/18/24				Negative		Negative	10/22/24	
							Negative	3/24/25	
							Negative	7/22/25	Sold as sterile 10/20/25.
364		12/ 1/23	Negative		Negative	Pigs died	Negative	12/28/23	
							Negative	4/12/24	
							Negative	8/ 9/24	
	10/27/24				Negative		Negative	3/24/25	
							Negative	7/22/25	
520	10/22/23				Negative		Negative	11/ 6/23	
							Positive	2/16/24	
							Positive	6/27/24	
	1/16/25				Negative		Negative	2/18/25	
							Negative	6/20/25	
		11/10/25	Positive		Positive				
523	1/ 2/23				Negative		Negative	3/ 6/23	This animal was vaccinated following recorded calving. Sold as *sterile* 3/6/24.
							Negative	7/24/23	
							Negative	11/ 6/23	
527	4/20/24				Negative		Positive	7/24/23	
							Negative	11/ 2/23	
							Negative	6/27/24	
132	1/16/25				Negative		Negative	3/28/25	Transferred from Control Group 7/30/23.
							Negative	7/25/25	
355	9/ 6/24				Negative		Negative	10/16/24	
							Negative	3/24/25	
							Negative	7/ 7/25	Sold as sterile 1/25/26.
366	1/30/25				Positive				Tuberculin reactor 2/5/25.
375	8/13/24					Retained			Died following metritis 9/22/24.
511	10/28/24				Negative		Negative	10/18/23	
							Negative	2/15/24	
							Negative	5/ 9/24	
	1/29/25				Negative		Negative	3/24/25	
373	3/ 3/25					Retained	Negative	5/ 7/25	
							Negative	7/ 7/25	
531		10/24/24	Positive		Positive		Positive	3/28/25	
378	2/15/26				Negative				
379	6/17/25				Negative		Negative	6/20/25	
							Negative	9/12/25	
533		5/25/25		Lost	Negative	Discharge			
534		8/26/25		Lost	Positive		Negative colostrum		

CHART NO. IV

Bacteriologic Data on the Control Group

Animal No.	Calved Normally	Aborted	Examination for the Presence of Bact. Abortus Bang in:						General Remarks
			Fetus		Placenta		Milk		
			Result	Remarks	Result	Remarks	Result	Date	
8	6/14/23	2/ /24			Negative		Negative	9/ 8/23	
							Negative	12/28/23	
				Lost					
101	1/28/23				Negative		Negative	3/10/23	
							Negative	7/28/23	
							Negative	11/ 2/23	
	1/23/24				Negative		Negative	3/ 7/24	
							Negative	7/23/24	
							Negative	10/ 3/24	
	12/ 8/24				Negative		Negative	2/20/25	
							Negative	7/10/25	
132	3/11/23				Positive		Negative	3/27/23	Transferred to vaccinated group on 7/30/23.
							Negative	6/15/23	
134		4/ 1/23	Positive		Positive		Negative	7/ 7/23	Slaughtered on 3/6/24. Culture of genital organs was sterile.
							Negative	11/ 2/23	
		12/ 7/23	Positive		Positive	Discharge	Negative	3/ 5/24	
138	1/23/23					No exam.	Positive	10/ 9/23	
		10/12/23	Positive		Positive		Positive	2/ 7/24	
							Negative	6/12/24	
		3/ /24		Lost		Lost			
	7/12/25				Negative		Negative	8/16/25	
141	6/13/23					Lost	Negative	9/ 8/23	
							Negative	2/15/24	
	7/13/24					Lost	Negative	7/23/24	
							Negative	11/ 8/24	
	6/25/25				Negative	Pigs died	Negative	7/10/25	
142		3/29/23	Negative	Twins	Negative		Negative	7/28/23	Sold as *sterile* 9/8/24.
							Negative	11/ 5/23	
							Positive	3/ 5/24	
143	11/ 4/23				Negative		Negative	1/15/24	This animal has never given serologic evidence of Bact. abortus Bang infection.
							Negative	5/21/24	
							Positive	10/ 3/24	
	1/ 4/25				Positive		Negative	4/18/25	
							Negative	7/25/25	
		12/10/25	Negative		Negative	Discharge			
152	12/ 8/23				Negative	Pigs died	Negative	1/15/24	
							Negative	5/21/24	
							Negative	10/16/24	
		2/28/25	Negative		Negative		Negative	5/ 7/25	
153	12/ 9/24				Negative		Negative	1/28/25	
							Negative	2/11/25	
		12/31/25	Positive		Positive				
154	5/15/24				Positive		Negative	7/23/24	
							Negative	2/11/25	
	1/12/26								
350	11/12/23				Negative		Negative	12/19/23	
							Negative	4/10/24	
							Negative	8/ 1/24	
	3/10/25				Negative		Negative	5/ 7/25	
							Negative	8/18/25	
	1/ 9/26								
354	12/ 7/22					Consumed	Negative	12/31/22	
							Negative	3/28/23	Reactor to tuberculin.
							Negative	8/31/23	
	6/11/24				Negative		Negative	8/ 1/24	Slaughtered 5/27/25.
							Negative	2/18/25	
358	8/ 4/23				Negative		Negative	8/21/23	
							Negative	12/19/23	
							Negative	4/13/24	
	10/22/24					Retained	Negative	4/ 9/25	
							Negative	8/ 7/25	
	9/ 4/25				Negative				
360	1/ 4/24				Negative		Negative	2/ 1/24	
							Negative	6/11/24	
							Negative	10/16/24	
		1/26/25	Positive		Positive		Positive	4/ 9/25	
368	3/ 8/25					Not Exam.	Negative	4/ 9/25	
							Negative	7/29/25	
		12/ 1/25	Negative		Negative		Negative	12/ 1/25	
374	3/ 8/23				Positive		Negative	3/28/23	On 3/13/25 a yellowish brown discharge from the vagina was examined for Bact. abortus Bang but failed to show the presence of this organism.
							Negative	8/31/23	
							Negative	12/28/23	
	3/20/24				Negative		Negative	4/30/24	
							Negative	8/12/24	
		12/13/24		Decomposed	Negative		Negative	12/13/24	
							Positive	4/17/25	
		10/21/25	Positive		Positive		Negative	10/21/25	
375		1/28/23	Positive		Positive				Transferred to vaccinated group on 7/30/23
376	2/16/24				Negative		Negative	4/16/24	
							Negative	8/12/24	
	5/20/25					Consumed	Negative	5/20/25	
							Negative	8/18/25	
377		1/30/24	Negative		Negative		Negative	4/16/24	
							Negative	8/ 9/24	
	2/15/25				Negative		Negative	2/15/25	
							Negative	7/29/25	
521		11/ 5/24	Positive		Positive		Negative	11/ 5/24	
							Negative	5/23/25	
	2/ 7/26				Negative				
524	12/13/24				Negative		Negative	12/13/24	
							Negative	4/17/25	
	11/ 3/25				Negative				
526	3/ 7/24				Negative		Negative	5/22/24	
							Negative	10/22/24	
	4/15/25				Negative		Negative	7/29/25	
							Negative	9/10/25	
528		2/ 8/23	Positive		Positive		Negative	4/ 8/23	
							Positive	11/23/23	
	2/10/24					Retained	Positive	3/10/24	
							Positive	8/27/24	
529	9/17/24				Negative	Pigs died	Negative	9/17/24	
							Negative	4/23/25	
	10/26/25				Negative				
535	1/ 3/25					Consumed	Negative	2/17/25	
							Negative	5/23/25	
536	6/ 2/25				Negative		Negative	6/ 5/25	
							Negative	8/13/25	
537		11/11/24	Positive		Positive		Negative	11/13/24	
							Negative	5/23/25	
		8/ 5/25	Positive		Positive		Positive	8/ 5/25	
19	8/26/24					No Exam.	Negative	10/22/24	
							Negative	4/ 9/25	
	12/ 5/25				Negative				
21	12/22/24					Retained	Positive	1/21/25	Never gave serologic evidence of infection.
							Negative	4/ 9/25	
							Negative	8/ 5/25	
369	11/30/24				Negative	Pigs died	Negative	12/ 5/24	
							Negative	4/18/25	
370	3/16/25				Negative		Negative	5/ 7/25	
							Negative	8/ 5/25	
372	3/ 1/25					Retained	Negative	5/ 7/25	
								8/ 5/25	

CHART NO. V

The Results of the Serological Tests on the Vaccinated and Control Groups

Animal No.	Reaction of Dam	November, 1920 Agglutination	October 1922 Agglutination	March 1923		October 1923		February 1924		September 1924		January 1925		May 1925		October 1925	
				Agglutination	Comp-fixation	Agglutination	Comp-fixation	Agglutination	Comp-fixation	Agglutination	Comp-fixation	Agglutination	Comp-fixation	Agglutination	Comp-fixation	Agglutination	Comp-fixation
12	++++++		------		+-+-+	++ P P--	+-+--	+++ P--	------	+++ P--	------	+++ P--	+-+-+	++ P---	------	+ P----	P----
132	------	------				++++++	+-+-+	++++++	+-+-+	++++++	+-+-+	++++++	+-+-+	++++++	++++-	++++++	++++-
135	+++---	------	------	++++++	+-+-+												
136	+++---			++++++	+-+-+	++++++	+-+-+	++++++	+-+-+	++++ P-	+-+-+	+++ P--	+-+-+	++++--	++++-	++++ P-	++++-
140	------		+++---	++++++	+-+-+	++++++	+-+-+	++++++	+-+-+	++++++	+-+-+	+++ + P-	+-+-+	++++++	++++-	++++ + P	++++-
144	+++---		++----	++++++	+-+-+	++++++	+----	++++++	+-+-+	++++ +-	+-+-+	++++ ++	+-+-+	+++++-	++++-		
145	+++---		------	++++++	+-+-+	++++++	+-+-+	++++++	+-+-+	++++++	+-+-+	++++ P-	+-+-+	++++ P-	++++-	+++ P--	++----
310		+++---	++----	+++----		++++ P-	++-+-	+++ P--	+-+--	++-----	+-+--	+++++-	+-+-+	++++--	++++-	++----	++ P--
351	+++---	------	------	+++----	+-+-+	+ P----	+- P--										
352	+++---	------	P-----	+++----	+-+-+	+ P P---	+-+---	+++ P--	+-+--	+ P-----	+-----	+++++ P	+-+-+	+++ P--	+++--	+ P----	+ P---
355	++++++	+++++-	+-----	++-----	-----	++++++	+-+-+	++ P---	+-+-+	+ P-----	------	+ P P---	+-+-+	++++ P-	++++-	P-----	++ P--
361	+++---		------	+++---	+-+-+	++++ P-	+-+-+	++++++	+-+-+	++++++	+-+-+	++ P P P P	+-+-+	++++++	++++-	++++++	++++-
362	+++---		P P----	++++++	+-+-+	++++++	+-+-+	++++++	+-+-+	++++++	+-+-+	+++ P--	+-+-+				
363	+++---		++----	++++++	+-+-+	+++----	+-+-+	+++----	+- P--	+ P-----	+-----	+------	+-+-+	++++++	++++-		
364	+++---		------	+++++	+-+-+	+++---	+-+-+	+++----	+-+-+	+++ P--	+----	+++ P P-	+-+-+	P P----	++++-	P-----	+ P---
366			------	------	-----	+++ P--	+-+-+	+++----	+-+-+	P-----	+-+-+	------	+-+-+				
373	------		------	------	-----	------	------	+++++	+++++	+-----	+++++	------	+++++	+++++ P	++++-	+ P----	Anti-comp.
375						+ P-- P+	+-+-+	++++++	+-+-+								
379								------	-----	++++++	+-+-+	+++++ P	+-+-+	++++++	++++-	+++ P--	+++--
511		++----	------	+ P----	-----	P P----	-----	+------	------	+ P P---	+----	++++ P-	+-+-+	P P----	-----	P P----	+ P---
520	++----		------	+++++	+-+-+	+++++	+-+-+	+++++	--+-+	++++++	--+-+	++++++	+-+-+	++++++	++++-	++++++	+++--
523			------	+++++-	+-+-+	++----	+-+-+	++ P---	+-+-+								
527			------	+++++	+-+-+	+++++	+-+-+	+++++	+-+-+	++++++	+-+-+						
531	------			------		------	-----	+++++-	+-+-+	++++++	+-+-+	++++++	+-+-+	++++ P P	++++-	++++++	++++-
533	------					------	-----	------	-----	------	-----	+++++ P	+-+-+	++++++	++++-	++++++	++++-
534	++++++							------	-----	------	-----	++++++	+-+-+	++++++	++++-	++++++	++++-
378	------					------	-----	------	-----	++++++	+-+-+	+ P----	+-+- P	++++++	++++-	+ P----	++ P P-
8		------	++++++	++++++	+-+-+	++++++	+-+-+	++++++	+-+-+								
19			------	------	-----	+++ P--	+-+-+	P P----	+-+--	+ P----	+----	P-----	+----	P P----	-----	+ P----	+----
21										------	-----	------	-----	------	-----	------	-----
101		+++---	------	+ P----	+-+-+	++----	+-+-+	+ P----	+-+-+	+++ P--	+-+-+	P-----	+-+--	+ P----	++++-	+-----	++----
132	------	------	+-----		+-+-+												
134	++++++		++++++		--+-+	++++++	+-+-+	++++++	+-+-+								
138	+++---	------	------	------	-----	++++++	+-+-+	++++++	+-+-+	++++++	+-+-+	++ P---	+-+-+	++++++	++++-	++++++	++++-
141	------		------	------	-----	------	-----	------	-----	------	-----	------	-----	------	-----	------	-----
142			------	++++++	+-+-+	++++++	+-+-+	++++++	+-+-+								
143	++----		P P----	------	-----	------	-----	P P----	-----	------	-----	------	-----	------	-----	------	-----
152						------	-----	------	-----	------	-----	------	-----	------	-----	------	-----
*153						------	-----	------	-----	------	-----	------	-----	------	-----	------	-----
154						+ P----	-----	++ P---	+-+--	P-----	+- P--	P-----	+- P--	P-----	-----	------	-----
350	+++---		++++++	+++++ P	+-+-+	++++++	+-+-+	++++++	+-+-+	+++++ P	+-+-+	++++ P P	+-+-+	++++ P-	++++-	+++ P P-	+++++-
354	+++---		------	+-----	-----	------	-----	++----	-----	------	-----	------	-----	------	-----	------	-----
358			P P----		-----	------	-----	+-----	-----	------	-----	------	-----	------	-----	------	-----
360			------	------		+ P P---	+-+-+	+-----	-----	------	-----	+++++	+-+-+	++++++	++++-	++++++	++++-
374						++++++	+-+-+	++++++	+-+-+	++++++	+-+-+	+ P P---	+-+-+	++++++	++++-	++++++	++++-
375						+ P-- P+	+-+-+										
376						------	-----	+-----	-----	------	-----	------	-----				
377						------	-----	------	-----	------	-----	------	-----	------	-----	------	-----
521	++----		P-----	++----	-----	+ P P P--	+-+-+	+++++ P	+-+-+	++++--	+-+-+	++++--	+-+-+	++++ P-	++++-	++++++	++++-
524			------	++ P---	+-+-+	------	-----	------	-----	------	-----	------	-----	------	-----		
526			------	++----	-----	+ P----	+-+-+	------	-----	------	-----	------	-----	------	-----	------	-----
528						++++++	--+-+	++++++	+-+-+	++++++	+-+-+	++++++	+-+-+	++++++	++++-	++++++	++++-
529						++++++	+-+-+	++++++	+-+-+	++++++	+-+-+	+++---	+-+-+	++++++	++++-	++++++	++++-
535								++++++	+-+-+	++++++	+-+-+	++++++	+-+-+	++++ P P	++++-	++++++	++++-
536								+ P----	+-+-+	------	+-+-+	------	+-+-+	------	++++-	------	++ P--
537								++++++	+-+-+	++++++	+-+-+	++++++	+-+-+	++++++	++++-	++++++	++++-
368						P P----	-----	P-----	-----	------	-----	------	-----	------	-----	------	-----

Note 153 Jan. 2nd, 1926. Agg = ++++++ Comp-fixation = ++++-